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Two new invasive hemipteran species first recorded in Romania: *Orientus ishidae* (Matsumura 1902) (Cicadellidae) and *Acanalonia conica* (Say 1830) (Acanaloniidae)

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Abstract

In the year 2016, two exotic invasive species of *Auchenorrhyncha*, the Asian leafhopper *Orientus ishidae* (Matsumura) (Cicadellidae) and the Nearctic planthopper *Acanalonia conica* (Say) (Acanaloniidae) were recorded for the first time in Romania. A number of 63 adult specimens of *O. ishidae* and 21 adult specimens of *A. conica* were trapped from July until September, using yellow sticky traps placed on cherry and apple orchards, common hawthorn and Chinese date trees in Bucharest (southern Romania), namely in northern area of the city in the fields of some research units, and in central area in the Botanical Garden 'D.Brândză'. Therefore, it is very important to know their further spreading and population density in our country in the near future, in order to evaluate their pest potential to crops, mainly those of economic importance, such as orchards, grapevine and ornamentals.

Keywords: *Orientus ishidae*, *Acanalonia conica*, invasive species, Romania

1. Introduction

In Romania, as in many other world countries, the research on invasive pest species has become a priority concern, which increases due to important practical and scientific interests. In the past few years, a lot of invasive species of new pests belonging to different groups of arthropods have been reported on the territory in Romania. Many of these were signaled in the southern parts of the country where they were firstly recorded for Romania. In most cases these were found on vegetation from the urban areas of the cities. For instance, in 2009 two Nearctic species of *Auchenorrhyncha*, the citrus flatid planthopper *Metcalfa pruinosa* Say (Flatidae) and the grapevine leafhopper *Scaphoideus titanus* Ball (Cicadellidae) were found, formerly in the urban areas of Constanța^[1] and latterly in Bucharest^[2], both cities in the southern part of Romania. In 2010 one species of Lepidoptera, the box tree moth *Cydalima perspectalis* Walk (Crambidae) was also found in Bucharest on Buxus ornamental plants^[3]. Three species of fruit flies, i.e. the Mediterranean fruit fly *Ceratitis capitata* L. (Tephritidae) on Chinese date trees, the spotted wing drosophila *Drosophila suzukii* (Drosophilidae) on wild black currant in 2013, and *Zaprionus tuberculatus* Malloch in 2014, were also firstly recorded for Romania in Bucharest area^[4-6]. Moreover, in 2015, the stinks bug *Halyomorpha halys* Stål was also recorded for the first time in Bucharest^[7].

The mosaic leafhopper species *Orientus ishidae* (Matsumura 1902) and the green cone-headed planthopper *Acanalonia conica* (Say 1830) are two alien invasive species from *Auchenorrhyncha* group that have been recently reported on the European continent, more precisely the former species in 1998 in Northern Italy^[8] and the latter ones in 2002 in Switzerland^[9]. The two species are originated from two different continents, Asia and Northern America, respectively. These belong to different taxonomic classification. *O. ishidae* is an Asian species belonging to the suborder Cicadomorpha, family Cicadellidae, subfamily Deltocephalinae, whereas *A. conica* is a North American species from the suborder Fulgoromorpha, family Acanaloniidae. Previously, *A. conica* was treated as the member of the subfamily Acanaloniinae, from the family Issidae. Presently, the subfamily Acanaloniinae has become the family Acanaloniidae^[10]. However, they have some common essential features, such as: they have one generation per year and survive the winter in an egg stage which lays in the woody tissue of the host plants; they are characterized by being extremely polyphagous

insects; they are considered serious pests of cultivated plants, affecting them in different ways; they are also known for having a good ability to adapt to new areas, so that both species are present in countries from Eastern Europe, after having spread from their initial infesting emergence points in Central Europe [11, 12].

The leafhopper *O. ishidae* represents a great risk because of its adults which are associated with plant pathogens such as the phytoplasmas of grapevine. In three important vine-growing countries in Europe, Slovenia [13, 14], Italy [15] and Switzerland [16], adults of this species were found infected with phytoplasmas of the 16SrV group, which causes the phytoplasmosis *Flavescence Dorée* (FD), a grave yellows - type disease for grapevine on the European continent. Moreover, they were found to transmit the phytoplasmas of the 16SrV group to vine plants [17].

With respect to the planthopper *A. conica*, its impact on plants is expressed by masses of white wax produced by larvae and honeydew secreted by larvae as well as adults [10] that cause serious damages to a wide spectrum of cultivated and spontaneous plant species, woody and herbal ornamental plants, fruit trees and vine [11].

This work aims at early detecting invasive species on Romanian territory in order to get important basically data

contributing to the knowledge related to their presence and power of spreading in the country. Within this work, we are able to provide the first information on the presence of the mosaic leafhoppers *Orientus ishidae* and the green cone-headed planthopper *Acanalonia conica* in Romania.

Material and Methods

The insect sampling of *O. ishidae* and *A. conica* in 2016, was conducted with yellow sticky traps in two type of habitats: urban, in northern and central areas of Bucharest and rural, in the Didactic Farm of the University of Agronomic Sciences and Veterinary Medicine Bucharest in Moara Domneasca (Ilfov County) at about 15 km away north of Bucharest, both in the southern part of Romania (figure 1). The urban areas included four sampled sites with six collecting points distributed in the areas of the Research-Development Institute for Plant Protection (RDIPP), the Research-Development Station for Fruit Growing (RDSFG), the University of Agronomic Sciences and Veterinary Medicine (UASVM), all placed in the northern part of Bucharest, and two collecting points in the Botanical Garden 'D.Brândza' situated in the middle of Bucharest.

The distribution of the insect collecting points is illustrated in figure 1.

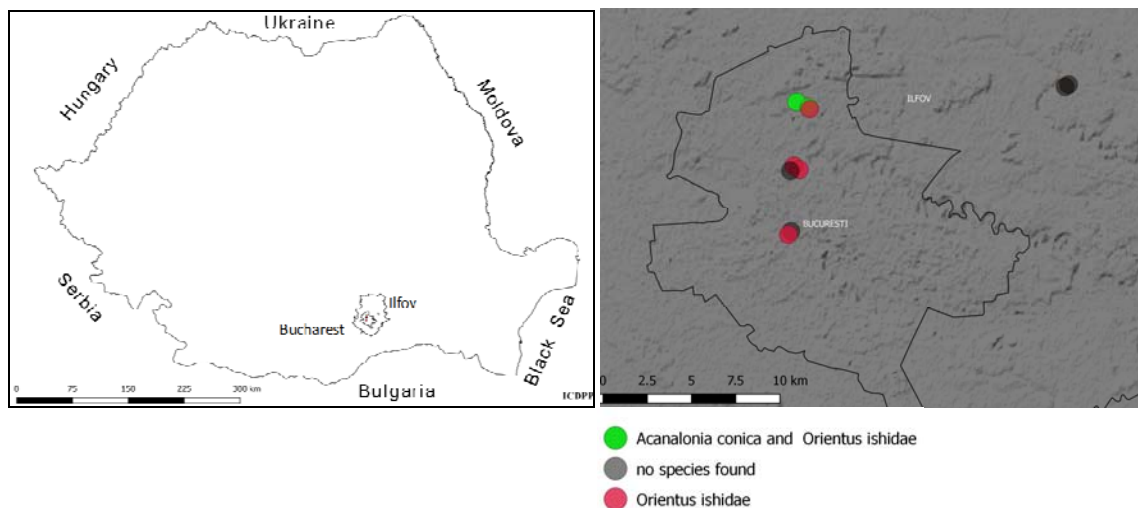


Fig 1: Insect collecting points on yellow sticky traps in areas from Bucharest and Ilfov County in 2016

Two-six yellows sticky traps of autochthonous production, AtraCERAS (20x30cm) were placed at each sampling site, on cultivated and spontaneous plants, during July 15th until

October 30th, 2016. Data on the investigated sites, the number of traps, the geographic position as well as the plant species which hosted the traps are shown in Table 1.

Table 1: Sampling sites, geographic coordinates, and plant species sampled to collect *O. ishidae* and *A. conica* in 2016

Sampling location/no of traps		Coordinates of sampling points	Plant species for traps hanging
RDIPP Bucharest	2 traps	44°30'15.6"/26°04'02.1"	<i>Crataegus monogyna</i> (Common hawthorn)
RDSFG Bucharest	3 traps	44°30'05.2"/26°04'35.3"	<i>Malus domestica</i> (Apple)
	3 traps	44°30'02.6"/26°04'37.0"	<i>Prunus avium</i> (Cherry)
UASVM Bucharest	1 trap	44°28'18.3"/26°03'58.7"	<i>Crataegus monogyna</i> (Common hawthorn)
	1 trap	44°28'11.6"/26°04'12.2"	<i>Ziziphus jujube</i> (Chinese Date)
Botanical Garden 'D.Brândza' Bucharest	2 traps	44°28'09.1"/26°03'49.2"	<i>Vitis vinifera</i> (Vineyard)
	1 trap	44°26'17.4"/26°03'52.9"	<i>Ziziphus jujube</i>
Didactic Farm of UASVM, Moara Domneasca (Ilfov)	1 trap	44°26'11.5"/26°03'46.5"	<i>Crataegus monogyna</i>
	2 traps	44°29'57.6"/26°15'32.2"	<i>Prunus domestica</i> (Plum)
	4 traps	44°30'12.0"/26°15'49.7"	<i>Juglans regia</i> (Walnut)

The traps were settled in the canopy of trees at about 1.7 m height from the ground. Checking and replacing the traps with

new ones were done every seven or ten days. To get alive specimens for photos, two samples of beating branches were

executed on hawthorn trees in RDIPP area and in the apple orchard of RDSFG in August.

The adult insects were examined and counted under a stereomicroscope Olympus SZ61 in the laboratory and the photos were taken using a digital compact Olympus camera attached to the above mentioned Microscope. The collected specimens were identified as belonging to *O. ishidae* and *A. conica* by the following descriptions of the key characters used in morphological diagnosis for the two species, as well as the illustrations presented in the published literature dedicated to them [12, 18-21]. The data according to which the leafhopper species in the genus *Orientus* which exist in European countries, were assigned to *O. ishidae* [8], have also been helpful in distinguishing members of this species in our samples. The genital armature of male specimens of *O. ishidae* has been examined conforming to the descriptions given by Guglielmino [8], and Biedermann and Niedringhaus [18]. The specimens of the two species are deposited at the

Institute for Plant Protection Bucharest.

Results

The adult specimens collected in surveyed areas in 2016, and examined as new invasive species, were found to correspond with descriptions of morphological characters and pictures provided by the literature in this field, for the species of *O. ishidae* and *A. conica*. The pictures for the specimens of *O. ishidae* collected in our samples are illustrated in Figure 2. The adults of *O. ishidae* were distinguished by their greyish yellow color, with characteristic mosaic-like designs of brown colour (or reddish in some cases) across the forewings, head, forehead, pronotum and scutellum; presence of an orange-printed model on the head between the eyes; the legs are dark with tarsus and tibia orange, excepting tibia of the hind pair legs. The characteristics of the male genital armature for our specimens corresponded with those presented in the reports of Guglielmino [8] and Biedermann and Niedringhaus [18].



Fig 2: Adults of *Orientus ishidae* on yellow sticky traps: (a) dorsal view; (b) lateral view

The specimens member of green cone-headed plant hopper *A. conica* were recognized based on the aspects of external morphology described and illustrated in the publications of Freund and Wilson [22], D'Urso and Uliana [20], Aldini *et*

al., [10], and Bartlett *et al.*, [19]. Adults of *A. conica* exhibited very distinctive green color; the specimens that are alive (Fig. 3a) have a bright green and the dead ones (Fig. 3b) become pale yellow color.

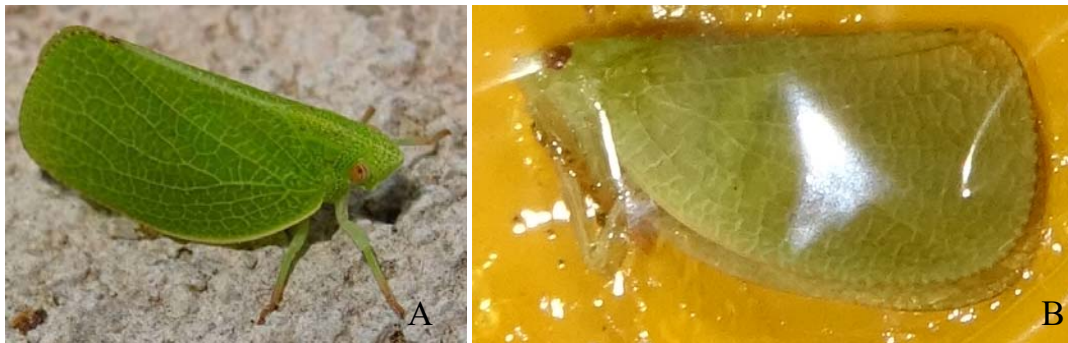


Fig 3: Adults of *Acanalonia conica*: (a) Alive adult of bright green color; (b) adult of pale yellow color on yellow sticky traps

The adults have the forewings rounded on ventral margins, and do not show parallel cross veins along the costal margin. They have a very prominent head, taking the form of a conical angle, a perfect reason for the name of this species: cone-headed planthopper.

The results of our collection on yellow sticky traps in 2016, confirmed the presence of the adults of *O. ishidae* and *A. conica* species in urban areas in Bucharest, and not in the rural area surveyed in Ilfov County, situated at 15 km away north of the city. Captures of the adults for both hopper

species, according to the sampling sites and host plant for yellow sticky traps are given in table 2. Two adults of *A.*

conica captured by beating shoots of *C. monogyna* were added to those trapped on yellow sticky traps.

Table 2: The number of adults belonging to *O. ishidae* and *A. conica* collected in the Southern Romania in 2016

Sampling location / no of traps	Plant species for traps hanging	<i>Orientus ishidae</i>	<i>Acanalonia conica</i>
RDIPP Bucharest	<i>Crataegus monogyna</i>	9	12
RDSFG Bucharest	<i>Malus domestica</i>	1	9
	<i>Prunus avium</i>	6	0
UASVM Bucharest	<i>Crataegus monogyna</i>	43	0
	<i>Ziziphus jujube</i>	1	0
	<i>Vitis vinifera</i>	0	0
The Botanical Garden 'D. Brândză' Bucharest	<i>Ziziphus jujube</i>	0	0
	<i>Crataegus monogyna</i>	3	0
Didactic Farm of UASVM, Moara Domneasca (Ilfov)	<i>Prunus domestica</i>	0	0
	<i>Juglans regia</i>	0	0
Total adults		63	21

The captures of the mosaic leafhopper *O. ishidae* on the yellow sticky traps amounted 63 adults, trapped in six out of the eight collecting points in the surveyed urban areas in Bucharest, on the traps placed on *Crataegus monogyna* (common hawthorn), *Malus domestica* (apple), *Prunus avium* (sweet cherry) and *Ziziphus jujube* (Chinese date). The number of specimens ranged from 1 to 43 adults per collecting point. Most adults (87.3%) of *O. ishidae* have been collected on the traps placed on *C. monogyna* trees existing in the areas of UASVM University and RDIPP Institute in the northern area of Bucharest, and the Botanical Garden 'D. Brândză' in the middle of the city. Six adults were trapped on *P. avium* and one adult was trapped on *M. domestica*, both plant species composed the fruit orchards of RDSFG Station, both chemical treated for pest insect and pathogens. Three adults of *O. ishidae* were found on the trap placed on *Z. jujube* in the Botanical Garden in the middle of Bucharest. No adult of *O. ishidae* was found on the traps placed in the plot of *V. vinifera* cultivated in the research area of Agriculture

University. This plot was also subjected to chemical treatments in programs of phytosanitary protection.

Regarding the green cone-headed planthopper, *A. conica*, its population density has counted 21 adults; 19 specimens on yellow sticky traps and 2 specimens obtained by beating branches of hawthorn in august. Its adults were found only in two out of ten collecting points, namely on the traps settled on common hawthorn in the area of RDIPP and in the apple orchard of RDSFG, both sites being placed close enough to one another, in the northern part of Bucharest. An abundant presence of *M. pruinosa* was also observed in the surveyed areas. Similar to *O. ishidae*, no specimens of *A. conica* were detected on the traps in the plot of *V. vinifera* in the area of Agriculture University.

Adults of both *O. ishidae* and *A. conica* species were collected from the middle of July until the middle of September (Fig 4).

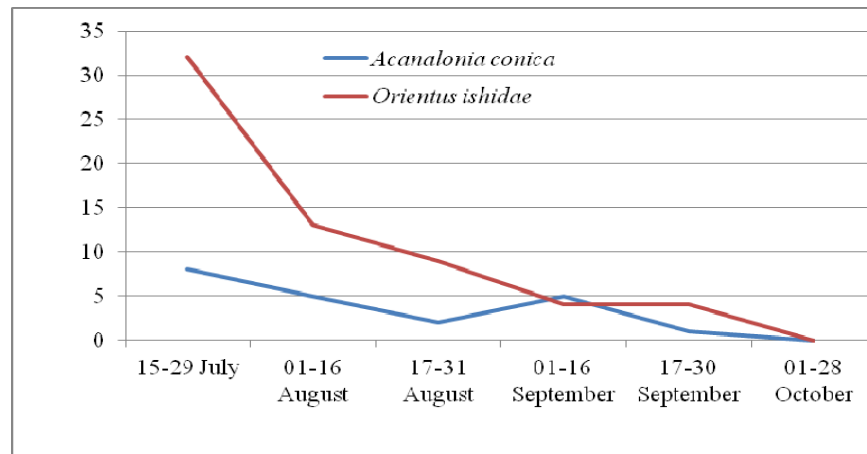


Fig 4: Seasonal collecting data of adults of *O. ishidae* and *A. conica* in 2016

In our study conducted in 2016, the first adults of *O. ishidae* were caught on 20th of July, on traps in the sweet cherry orchard of RDSFG Station as well as on a common hawthorn tree in the botanical garden of the Agriculture University. The first adults of *A. conica* on traps appeared on 29th of July on hawthorn in the RDIPP area and in the apple orchard of RDSFG.

On the 16th September, there were recorded the last captures of the two species; *O. ishidae* on the traps on common hawthorn in the botanical garden of the Agriculture

University on the one side, and *A. conica* on the traps from the apple orchard of RDSFG, on the other side.

With respect to the species of plants which hosted the yellow sticky traps, the trees of common hawthorn provided the most leafhopper *O. ishidae* on traps, i.e. a number of 55 specimens corresponding to 87.3% were collected on the settled traps. As for *A. conica*, out of 21 captures, almost half of adults were trapped on common hawthorn and the rest of adults on apple trees.

Discussion

The results of our survey carried out in 2016 clearly confirmed the presence of two invasive species from *Auchenorrhyncha* group, namely the mosaic leafhoppers *Orientus ishidae* and the green planthopper with conic head *Acanalonia conica*, on the Romanian territory, namely in areas in Bucharest, the Southern part of the country. It is important to note that, out of the two urban and rural habitats, monitored by us with yellow sticky traps in 2016, the adults of *O. ishidae* and *A. conica* were captured only on sites in urban areas, distributed in the northern and central parts of Bucharest. No adult was found in the surveyed rural area of Ilfov County. Our results are in accordance with the data reported in the literature for these species, mentioning that the first specimens of *O. ishidae* as well as *A. conica* were generally detected in many European countries, mainly in the urban areas, on ornamental or other species of trees. For instance, the first report of *O. ishidae* for Italy^[8], Slovenia^[23], Czech Republic^[24] and Hungary^[12] were in urban areas within the big cities, as Milano, Nova Gorica, Brno or Budapest. In a similar way, the acanaloniid *A. conica* was first detected in Europe in the urban habitats of Venetia^[11].

In our study, the adults of the two species were collected on the yellow sticky traps from the second decade of July to the middle of September. Although the yellow sticky traps were left active until the end of October and periodically checked out, no adult of *O. ishidae* or *A. conica* has been captured by the end of the sampling period in October. Here it must be added that in the period after the second decade of September, the climatic weather in surveyed areas was hostile, with severe decreases in the level of daily average temperature (6–11°C) associated with persistent rainfall. The unfavorable weather may be considered to be responsible for our inability to catch other specimens, after the middle of September.

The adults of *O. ishidae* appeared on the traps in the third week of July and continued till the middle of September. The data of Koczor *et al.*^[12] in Hungary showed a shorter active period of *O. ishidae* adults until the half of August, whereas the data of Lessio *et al.*^[17] in Italy indicated a longer period to the half of October. Compared to *O. ishidae*, the adults of *A. conica* were firstly trapped one week later in July. The capturing data referring to *A. conica* are in accordance to those published for this species by the literature in Italy^[10,11].

The number of adults was different from one collecting date to the other. Despite the fact that the adult population of the two species had a relatively low level, it could be noticed a seasonal trend in their dynamics (Fig 4). The seasonality of the adult captures can indicate the fact that both hemipteran species had a single generation a year, as is published in the literature. Based on the fact that, the areas investigated in 2016 were previously surveyed with yellow sticky traps, and none of the two species have been confirmed, we could deduce that *O. ishidae* and *A. conica* emerged in Bucharest area in 2016.

Both *O. ishidae* and *A. conica* are characterized as polyphagous species living on numerous plant species, such as trees, vine, shrubs and grasses. Among the host plants preferred by *O. ishidae*, there are indicated *Gleditsia triacanthos*, *Quercus palustris*, *Malus domestica*, *Prunus domestica*, *P. virginiana*, *Rubus fruticosus*, *Rosa canina*, *Crataegus oxyacantha*, *Juglans regia*, *J. nigra*, *Berberis spp.*, *Buxus sempervirens*, *Chelidonium majus*, *Vitis vinifera*, *Viburnum tinus*, *Viburnum sp.*, *Ulmus minor*, *Salix spp.*, *Hedera helix*, *Cornus sanguinea*, *Betula spp.*, *Acer spp.*, and *Carpinus spp.*^[8, 12, 21, 23]. As well, *A. conica* is widely reported

in literature as being associated with many wild and cultivated plants species, such as *Amorpha fruticosa*, *A. conicawasa*, *Corylus avellana*, *Buddleja davidii*, *Cornus sanguinea*, *Prunus sp.*, *Urtica dioica*, *Parietaria officinalis*, *Humulus lupulus*, *Solanum nigrum*, *Chenopodium sp.*, *Xanthium italicum*, *Rubus sp.*, *Ulmus sp.*, *Morus sp.*, *Robinia pseudoacacia*^[10,20].

In our survey, the adults of *O. ishidae* were caught on yellow sticky traps placed on *Crataegus monogyna* (common hawthorn), *Malus domestica* (apple), *Prunus avium* (sweet cherry) and *Ziziphus jujube* (Chinese date) whereas the adults of *A. conica* were captured only on yellow sticky traps on *C. monogyna* and on *M. domestica*. No adult of *O. ishidae* or *A. conica* was trapped on the grapevine plot investigated in the surveyed area. Also, neither of the two species was trapped on the yellow sticky traps placed in the plum and walnut orchards in the surveyed area located in Ilfov County, near Bucharest city.

At this time, because of the low population density, the two species of *Auchenorrhyncha* have not showed any impact on the plants in collecting areas. But, considering the remarks published in the specialized literature^[17, 20, 25, 26] related to the rapid capacity of the two insects to spread in European countries, we expect that these to develop significantly in the years after their first appearance, and, consequently, cause damages to plants in our country. Among the plants of economic importance, the grapevine represents the main target crop to be damaged in the case of *O. ishidae*, and the ornamental plants in the case of *A. conica*. The mosaic leafhopper *O. ishidae* was reported to be vector of the phytoplasmas associated with X-disease of stone-fruit trees^[27] and with the grapevine yellow disease *Flavescence dorée*^[17]. The adaptability of this species to develop in climate conditions in Romania will be a very important cause of concern, because there are large areas of vineyards which will be their perfect target to attack. Its biology is likened to that of the grapevine leafhoppers *Scaphoideus titanus* (Cicadellidae, Deltocephalinae), a very dangerous insect that spreads the *Flavescence dorée* diseases in grapevines^[28].

With reference to *A. conica*, this develops a life cycle similar to that of the related species, the citrus flatid planthopper, *Metcalfa pruinosa* (Say 1830) (Fulgoroidea, Flatidae)^[20]. Thus, we expect that *A. conica* to colonize the plants from diverse habitats in a same way as *M. pruinosa*.

The mosaic leafhoppers *O. ishidae* as well as the green conic-head planthopper *A. conica* are reported as exotic species involuntarily introduced in Europe by human activity^[17, 20, 24]. A similar in-going of the two species could also be assumed in the observed areas in Bucharest. They may have been introduced into urban areas, possibly along with infested planting material used to re-generate the green spaces of the city, especially as a result of an intensive import of large amounts of ornamental plants that have been introduced for this purpose. From emergence points, the adults probably migrated to other plants like fruit orchards and other plants near them, i.e. common hawthorn trees, as we managed to collect in 2016.

Conclusions

The sampling results obtained in our field survey carried out in 2016 clearly confirmed the presence of two new invasive species of insects from *Auchenorrhyncha* group, namely the Asian leafhopper *Orientus ishidae* (Matsumura) (Cicadellidae) and the Nearctic planthopper *Acanalonia conica* (Say) (Acanaloniidae) in sites in the urban areas of the

northern and central parts of Bucharest city (Southern Romania).

The two species are expected to have an important impact on agriculture because they can produce serious damages to plants, directly by sucking for feeding, as well as indirectly, by transmitting systemic pathogens such as phytoplasmas (*O. ishidae*) or by producing masses of white wax and secretion honeydew that reduce physiological functions of plants (*A. conica*).

We consider that our results on the two invasive species *O. ishidae* and *A. conica*, which have first been determined in Romania, may offer valuable information and can represent new contributions to the previous knowledge on the invasive arthropods available in Romania and at European level as well, thus completing those which have been attained by now.

These data may be useful for good agricultural practices in plant protection, especially for horticultural plants of economic importance (i.e. grapevine, ornamentals). Accordingly, it is very important to perform further extended observations within an early detection program in order to evaluate the present spreading and population density of the two invasive species in the agro-ecosystems under specific climatic and agronomic conditions in Romania.

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